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Short Communication

PCR Assay for Identification of Animal Species in Different Ready to Eat Raw Meat Samples [1,2]

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Abstract

In this study, 500 ready to eat raw meat samples (minced meat, lahmacun ingredients, kebap, stew and meatball samples)analyzed for different animal originated DNA residues (pork, chicken, cattle, sheep, horse, donkey, cat, dog, mouse, cockroach and house fly) by PCR procedures. Besides, all the samples were analyzed for important foodborne pathogens (coliforms, *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes* and *Salmonella spp.*). According to the results, total of 52 samples were determined as adulterated and different originated animal DNA samples were found (chicken, horse and sheep DNA residues). Adulterated samples were also determined more risky for the consumers in microbiological aspect.

Keywords: *PCR, Species identification, Ready to eat meat products, Foodborne pathogens*

Tüketime Hazır Farklı Çiğ Et Örneklerinde PCR Prosedürleri ile Farklı Hayvan Türlerinin Araştırılması

Özet

Bu çalışmada 500 adet tüketime hazır çiğ et örneği (kıyma, lahmacun iç malzemesi, kebap) toplanılmış ve örnekler 9 adet farklı hayvana ait (domuz, tavuk, sığır, koyun, at, eşek, kedi, köpek, fare, hamamböceği ve sineği) DNA örnekleri PCR prosedürleri kullanılarak araştırılmıştır. Yanı sıra, her bir örnek halk sağlığı açısından risk teşkil edebilecek olan 5 adet gıda patojeni açısından (koliformlar, *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes* ve *Salmonella spp.*) analiz edilmiştir. 52 adet örnekte farklı hayvan türlerine ait (tavuk, at ve koyun olmak üzere) DNA kalıntıları saptanmıştır. Tüm taklit ve tağşişe maruz kalmış örnekler mikrobiyolojik olarak tüketici sağlığı açısından riskli olarak değerlendirilmiştir.

Anahtar sözcükler: *PCR, Tür tayini, Tüketime hazır çiğ et ürünleri, Gıda kaynaklı patojenler*

INTRODUCTION

The composition of food is a major concern of consumers today. In the case of adulterated meat product consumption, several factors including economic, food safety (allergy) and moral reasons (religious belief), trigger such apprehensions. Among these concerns, consumers are most sensitive because of religious factors and do not tolerate even trace amounts of adulteration of meat products with forbidden meats like pork [1]. Hygiene and right labeling notified on the label of any food stuff are very important criteria especially for public health.

This study aimed to examine various meat and meat products (kebaps, lahmacun ingredients, minced meat, stews, various meat balls etc.) which are presented in various sales points (restaurants, butcher shops, groceries etc.) in Istanbul region, to determine their ingredients

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through DNA typing method and to specify the different animal tissues/residuals in these products. Besides, all of the samples are checked for the 6 primary foodborne pathogens which can pose serious microbiological threats for consumers' health. The differences between adulterated and not adulterated products are determined by statistical methods.

MATERIALS and METHODS

Specimen Handling

Random sampling method has been used in this study. From 500 different sales points in the Istanbul region 500 meats and meat product samples have been collected.

Microbiological Analyses

The number of TAB was defined in Plate Count Agar (Oxoid, CM0325), coliforms in VRB (Oxoid, CM1082), *E. coli* in Tryptone Bile X-Glucuronide Medium Agar (Oxoid, CM0945), *S. aureus* in Baird-Parker Agar (Oxoid, CM0275) and DNASE Agar (Oxoid CM0321), *Salmonella* spp. in Xylose Lysine Desoxycholate Agar (Oxoid, CM0469) and Hectoen Enteric Agar (Oxoid, CM0419), and *L. monocytogenes* in Chromogenic Listeria Agar (ISO) Base (Oxoid, PO 5183) and Chromogenic Listeria Selcetive Supplement (ISO) (Oxoid, SR0226) and Oxford (Oxoid, CM856) and Palcam Agar (Oxoid, CM877) respectively to ISO 16649-2 2001, 4833 2003, 6888-1/A1 2004, 11290-1/A1 2005 and 6579/A1 2006 [2-7].

PCR

DNA of all isolates were extracted according to the protocol of the manufacturer (Macherey-Nagel, Nucleospin® Tissue). All the extracts were stored at -20°C until they are used as target DNA for the PCR procedure.

Statistical Analysis

In order to study the risk differences among adulterated and non–adulterated samples upon the studied microbiological parameters and to determine the statistical significance of these, Pearson correlation analysis has been used [8].

RESULTS

18 (3.6%) of the samples showed chicken DNA, 33 (6.6%) of them showed sheep DNA and 1 (0.2%) of them showed horse DNA. None of them showed pork, donkey, cat, dog, mice, cockroach and fly DNA. The detailed refraction of the results can be seen in *Table 1*. The positive results have been determined through Real-time PCR procedures.

The microbiological results are given in *Table 2*. According to coliform bacteria indications 41 (%8.2) of the samples, according to *E. coli* parameter indications 23 (4.6%) of the samples, according to *S. aureus* parameter indicators 29 (5.8%) of the samples, according to *L. monocytogenes* indications 8 (1.6%) of the samples, according to *Salmonella spp.*, 3 (0.6%) of the samples have been determined as unfit for human consumption. 70.3% of coliforms, 58.7% of *E. coli*, 72.4% of *S. aureus* and 100% of *Salmonella* spp. and *L. monocytogenes* detections are found in the adulterated samples.

DISCUSSION

In many countries, food fraud and adulteration in food products, especially in meat and meat products are done either deliberately in order to increase the profit margin or involuntarily as a result of not following the food safety standards, especially in facilities which process more than one animal species.

The main ingredient of kebap in our country is mutton and many kebap shops prepare their kebaps from a mixture of bovine meat and mutton; however, mixing meat products of different animal species either deliberately or accidentally poses a microbiological threat for the consumers, causes the consumers to consume meat products beyond their information. As a result, the consumer is deceived and retrospective follow-up, which is a very important part of food safety procedures, becomes too difficult. It is possible that especially the products containing different types of meat are deliberately adulterated or the facilities producing these in deliberately mingle different meat products.

Table 1. *Extraneous DNAs (other than cattle DNA) determined ın the samples*
Tablo 1. *Örneklerde gözlenen yabancı DNA'lar (sığır DNA'sının dışında)*

Region	Sample (raw)	Sales Point	Extraneous DNA	DNA Positive Samples
Istanbul Europe - İstanbul Asia	Lahmacun ingredients	Kebab shop	Chicken	11
İstanbul Europe - İstanbul Asia	Minced meat	Butcher shop	Chicken	5
İstanbul Europe	Kebap	Kebab shop	Chicken	2
İstanbul Europe - İstanbul Asia	Kebap	Kebab shop	Sheep	30
İstanbul Europe	Minced meat	Butcher shop	Sheep	3
Istanbul Asia	Minced meat	Butcher shop	Horse	1
TOTAL				52

Table 2. *Adulterated and unadulterated product differences according to the risks they pose for consumer health (Pearson Chi Square Method). The results show the difference between all the inadulterated samples and adulterated ones*

Tablo 2. *Tağşiş yapılan ve tağşiş yapılmayan et ürünleri arasındaki grup farklılıklarının tüketici sağlığını riske etmesi açısından analiz edilen mikrobiyolojik parametreler için sınanması (Pearson Chi Square yöntemine göre). Tablodaki sonuçlar tağşiş yapılmadığı tespit edilmiş tüm örneklerin toplamı ve tağşiş yapılmış et ürünleri arasındaki grup farklılıklarını yansıtmaktadır*

Statistical Methods	Microbiological Parameter	Relevant Variable	Value	Asymp. Sig
Pearson Chi Sq	Coliforms	Samples confirmed for adulteration/samples which don't have adulteration	11.087	.000
Pearson Chi Sq	*Escherichia coli*	Samples confirmed for adulteration/samples which don't have adulteration	1.05	.000
Pearson Chi Sq	*Listeria monocyotgenes*	Samples confirmed for adulteration/samples which don't have adulteration	12.102	.000
Pearson Chi Sq	*Staphylococcus aureus*	Samples confirmed for adulteration/samples which don't have adulteration	2.787	.000
Pearson Chi Sq	*Salmonella* spp.	Samples confirmed for adulteration/samples which don't have adulteration	3.902	.000

Values written in red are statistically relevant because they are smaller than P<0.005;Values written in red are positive for adulteration with regard to positive correlation. Adulterated meat and meat products pose a greater microbiological risk for consumer health than non-adulterated products

Medical literature states that some strains such as *S. aureus* are not very competitive and if their initial counts are lower, they cannot develop properly and their development is easily depressed in mixed cultures. Besides, lactic acid bacteria in the microflora of fermented foods and the antimicrobials they produce like the lactic acid, hydrogen peroxide and bacteriosin suppress pathogens such as *E. coli, S. aureus, L. monocytogenes and B. Cereus* [9]. It is thought that the staff hygiene practices are deficient in the facilities from which the *S. aureus* positive samples have been collected and this is the primary reason of these results.

The adulteration practices pose another risk which is often overlooked but actually important, that is food intolerance. The exogenous substances which are mixed in the adulterated products and the ingredients which might be different from the label information may cause the consumers to develop food intolerance reactions. This is considered one of the main risks of adulteration. Food intolerance may have various reasons. The prevalence of food intolerance reactions against foods and food additives is much higher than food allergies which include an immunological mechanism. Whatever the reason of the adulteration maybe, it results in deficient hygiene conditions and this is a serious threat for the facility, staff and product and consumer health. Besides, micro-organisms which reproduce in meat and meat products because of hygiene deficiency can quickly develop single or multi resistance to antibiotics through complex genetic interactions. Our study shows that adulterated products pose a statistically meaningful higher risk for consumer health than unadulterated products. Total quality management systems and food safety practices should be applied together with the official inspection of the state authorities; programs to raise consumer awareness and continuous training programs for the staff responsible for food production should also be carried into effect. All these would be beneficial to reduce the incidence of the adulteration practices.

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